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Studies on the Resalting of Salt-Cured Calfskins

When salt-cured hides or skins begin to show evidence of decomposition it is a common practice to resalt them in an attempt to prevent further deterioration. There is no quantitative information in the literature by which to judge how effective such a resalting operation may be. The experiments described herein were designed to determine, under controlled laboratory conditions, the effect of varying amounts of excess salt on bacterial multiplication and skin protein deterioration during storage.

Four salt-cured calfskins each weighing from 8 to 10 pounds were selected from the pack of a local butcher. The excess salt adhering to the hair and flesh sides was removed with a brush. The hair was then cut as closely as possible with electric clippers and a portion from the bend area of each skin was immediately diced into about ⅛-inch cubes. Each diced sample was thoroughly mixed and bottled. Duplicate 5-gram portions were taken for the determination of moisture and salt. Moisture was determined by drying in an oven at 60° C. for 48 hours. Salt was determined by digesting the dried samples in hot, concentrated nitric acid in the presence of a standard excess of 0.1*N* silver nitrate followed by titration with 0.1*N* sodium sulfocyanate in the presence of a ferric indicator. Salt-free, moisture-free skin was taken as the difference between 100 and the sum of the percentages found for salt and moisture. The results of these analyses are given in Table I.

TABLE I.

COMPOSITION OF SALT-CURED CALFSKINS

Cured Skin	Skin* per cent	Moisture per cent	Salt per cent
A	37.8	48.3	13.9
B	33.5	52.5	14.0
C	32.5	54.7	12.8
D	31.4	56.4	12.2

Twelve 10-gram samples each of diced skins A, B, C, and D were weighed into wide-mouth 100-ml. volumetric flasks. Sodium chloride (C.P. fine crystals) was then added in amounts of 0.0, 0.3, 0.6, 0.9, and 1.2 grams, respectively, to duplicate flasks of samples A, B, C, and D. The salt was thoroughly mixed with the diced skin by agitating the flasks. These flasks were fitted with cork stoppers, sealed with paraffin, and placed in an incubator at 30° C.* for six weeks. The purpose of sealing the flasks was to

maintain a constant moisture content during the incubation period since previous studies[1] have shown that the moisture content of salt-cured calfskin exerts a controlling influence upon the extent of microbial deterioration during storage.

This left duplicate samples from each skin for unincubated controls. To these controls 50 ml. of distilled H_2O was added respectively, and the flasks stored at 7° C. for 24 hours to allow all the pieces of skin to approach the same state of hydration. The flasks were then brought to volume with distilled water and three 0.01-ml. aliquots were removed from each to determine the number of bacteria present. Counting was done by the direct microscopic method, as described in a previous publication[1].

TABLE II

Bacterial count and amount of total soluble nitrogen, ammonia nitrogen, and carboxyl groups in salted calfskin before and after incubation for 42 days at 30-34° C.

Cured Skin	Sample No.	Composition of Incubated Sample			Period of Storage at 30-34° C.	Bacterial Count†		Total Soluble Nitrogen as Equivalent ml. of 0.1N HCL†	Ammonia Nitrogen as Equivalent ml. of 0.1N HCL†	Carboxyl Groups as Equivalent ml. of 0.1N KOH†
		Skin	Moisture	Salt		Average Count	Probable Error**			
		per cent	per cent	per cent	days	millions	millions			
	C*	37.8	48.3	13.9	0	126	9	3.17	0.32	1.72
A	1	37.8	48.3	13.9	42	129	8	3.17	0.46	1.73
	2	36.7	46.9	16.4	42	138	9	3.19	0.41	1.72
	3	35.6	45.5	18.9	42	104	8	3.15	0.41	1.73
	4	34.7	44.3	21.0	42	120	8	3.15	0.39	1.73
	5	33.7	43.1	23.2	42	101	8	3.17	0.42	1.73
	C*	33.5	52.5	14.0	0	66	8	4.69	0.92	1.43
B	1	33.5	52.5	14.0	42	1,260	142	8.36	1.36	2.15
	2	32.5	50.9	16.6	42	1,059	134	8.08	0.98	1.67
	3	31.6	49.5	18.9	42	1,095	138	7.35	1.07	1.43
	4	30.7	48.2	21.1	42	1,010	126	8.45	1.14	1.67
	5	30.0	47.0	23.0	42	1,325	146	8.54	1.39	1.79
	C*	32.5	54.7	12.8	0	312	52	4.44	0.73	1.37
C	1	32.5	54.7	12.8	42	2,463	216	8.48	1.39	3.48
	2	31.6	53.1	15.3	42	2,118	197	7.92	1.19	2.90
	3	30.7	51.6	17.7	42	1,358	186	7.35	1.14	2.61
	4	29.8	50.2	20.0	42	1,817	198	7.67	1.19	2.98
	5	29.0	48.8	22.2	42	1,935	208	8.41	1.36	3.19
	C*	31.4	56.4	12.2	0	609	94	3.92	0.52	1.03
D	1	31.4	56.4	12.2	42	3,371	225	9.34	2.42	3.50
	2	30.5	54.8	15.7	42	2,124	180	8.48	1.63	2.94
	3	29.6	53.2	17.2	42	1,919	203	8.67	1.43	2.94
	4	28.8	51.8	19.4	42	1,935	207	9.20	2.93	3.18
	5	28.0	50.3	21.7	42	4,245	293	11.07	3.49	3.71

The contents of each flask was then filtered through cheesecloth and the filtrate was brought to a 100 ml. volume. Aliquots of 25 ml. each were removed for the determination of total soluble nitrogen by the K.G.A. method and ammonia nitrogen by magnesium oxide distillation. Aliquots of 10 ml. each were also removed for titration of free carboxyl groups by the method of Willstatter[2], using 0.1*N* alcoholic potassium hydroxide with o-cresolpthalein as an indicator. At the conclusion of the incubation period of six weeks, each incubated sample was subjected to the same analytical procedure employed above with the unincubated controls. The results obtained are presented in Table II.

In this table samples numbered 1, 2, 3, 4, and 5 designate the incubated samples from each skin to which 0, 0.3, 0.6, 0.9, and 1.2 grams of salt had been added, respectively. The results given for each incubated sample and unincubated control are averaged values from duplicate determinations. The addition of increasing amounts of salt brought about a progressive decrease in the percentages of skin substance and moisture in each series of samples. The extent of these changes in percentage composition has been calculated for each skin and these values are also given in Table II.

The correlation obtained between direct microscopic bacterial counts and the extent of the chemical changes observed is plainly indicated in Table II. With skins B, C, and D there are large increases in the count with all of the incubated samples. In terms of the number of bacterial generations the differences in these increases are not large. Nevertheless, the arithmetical mean values considered together with the probable errors show that increases in the number of bacteria occurred in concordance with increases in the total soluble nitrogen, ammonia nitrogen and free carboxyl groups. Thus, it would appear that the chemical changes observed should be attributed to bacterial growth. In Figures I and II the probable error was added or subtracted to selected counts to emphasize the apparent direct relation existing between bacterial count and chemical changes. It would be permissible, though less logical, to employ the probable error in a similar manner to minimize this relationship. If the latter procedure was adopted the chemical changes could then be attributed to the effect of salt and moisture on the activity of the extracellular bacterial enzymes. With the direct microscopic method of counting no differentiation is made between living and dead cells. Therefore, the counts show all the multiplication that occurred during the incubation period of 42 days and would not necessarily indicate the number of living bacteria at the time the sample was taken.

In the salt-curing of hides and skins a great deal of emphasis has been placed on the proportional quantities of water, skin substance, and salt. In this study the original ratio of salt to skin substance in the four skins selected was approximately the same, about 0.4. The ratio of water to skin substance was 1.3 for skin A. 1.5 for skin B. 1.7 for skin C, and 1.8 for skin D.

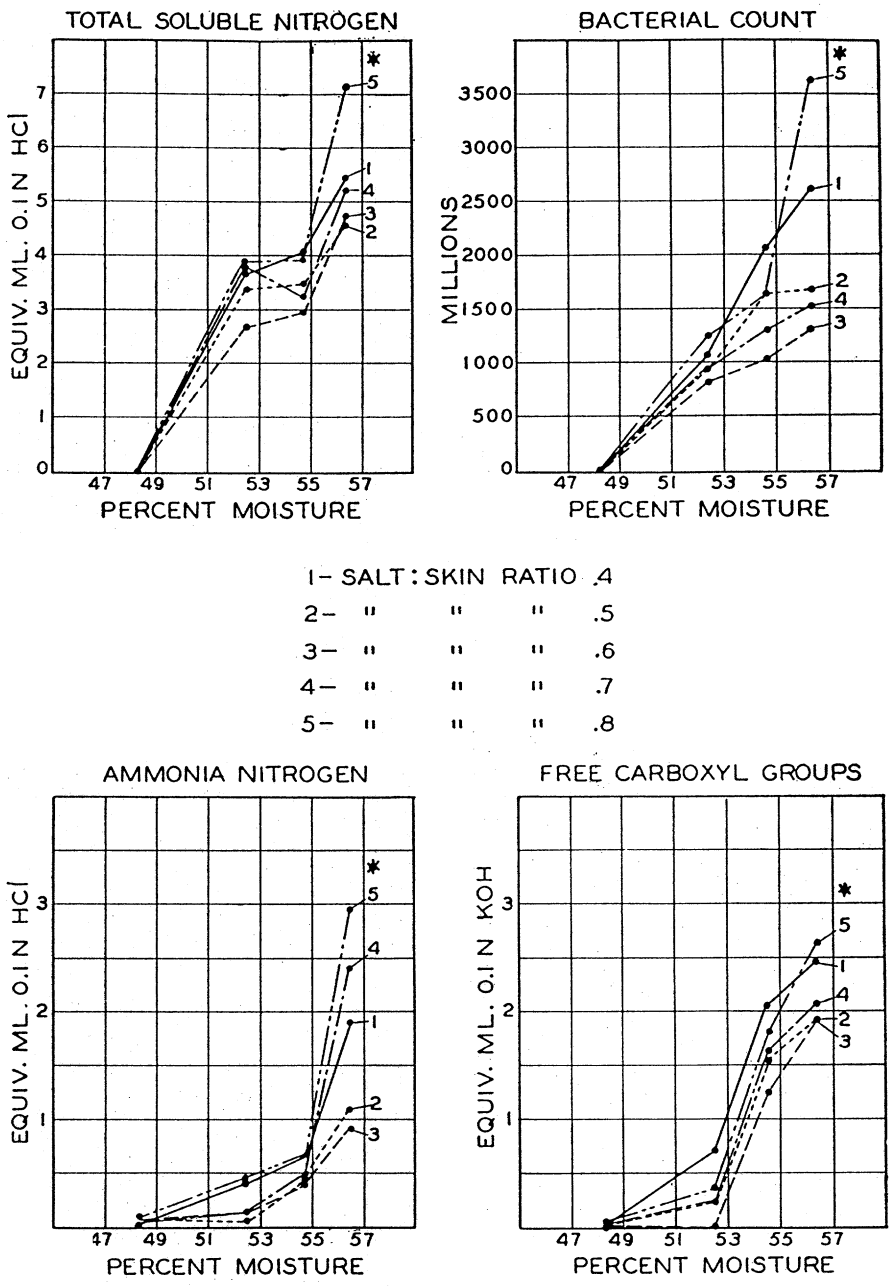

FIGURE I. Effect of moisture content of cured calfskin prior to resalting on increases in bacterial counts, free carboxyl groups, total soluble nitrogen and ammonia nitrogen.

The ratio of water to skin substance was constant for all of the incubated samples of each skin, whereas the ratio of salt to skin substance increased progressively in each series from 0.4 for the unincubated controls and the incubated samples designated by 1 in Table II to 0.5 for the incubated samples designated by 2, 0.6 for the samples designated as 3, 0.7 for those designated as 4, and 0.8 for those designated as 5. In Table III there is given the increase in total soluble nitrogen, ammonia nitrogen, free carboxyl groups, and bacterial count of the incubated samples over the unincubated controls as calculated from the data in Table II.

In the case of skin A, no bacterial growth or appreciable increase in total soluble nitrogen, ammonia nitrogen, or free carboxyl groups that might indicate activity of residual bacterial enzymes in the absence of bacterial growth, occurred during incubation. The initial moisture content of this skin was but 48.3 per cent, with a water-to-skin ratio of 1.3. Skins B, C, and D had initial moisture contents of 52.5, 54.7, and 56.4 per cent and water-to-skin ratios of 1.5, 1.7, and 1.8, respectively. Upon incubation, portions of these skins showed marked increases over the controls in bacterial count, total soluble nitrogen, ammonia nitrogen, and the number of free carboxyl groups. These results provide additional evidence confirming data previously published[1] relative to the limiting effect of a critical moisture content of salt-cured skin on microbial deterioration.

With the samples from skins B, C, and D the addition of excess salt did not prevent increases in the bacterial count, the number of free carboxyl groups, and the amounts of total soluble nitrogen and ammonia nitrogen, even though the maximum amount of salt added to both skins B and C was great enough to lower the percentage of moisture in the flasks of No. 5 samples to 47.0 and 48.8 per cent, respectively, (See Table II). Thus, the addition of excess salt failed, in these studies, to bring about a dehydration of the cured skin great enough to inhibit microbial deterioration. From the data presented in Table III it can be seen that where the ratio of salt to skin was 0.4 or higher, spoilage due to microbial activity occurred only when the ratio of water to skin substance was greater than 1.3, and the extent of bacterial growth with its resulting hydrolytic decomposition of skin substance became greater as this ratio increased to 1.8.

Where the ratio of water to skin substance was 1.5 or higher, increases in the ratio of salt to skin substance from 0.4 to 0.8 resulted in greater preservative efficiency as this ratio increased to 0.6. With further increases a decrease in preservative efficiency actually occurred. No evidence is shown that suggests that ratios higher than 0.6 for salt to skin substance improved the efficiency of preservation, even when the water to skin substance ratio was increased to 1.8.

From the standpoint of practical resalting operations, the above results suggest that where salt alone is used an excess of about 5 or 6 per cent over

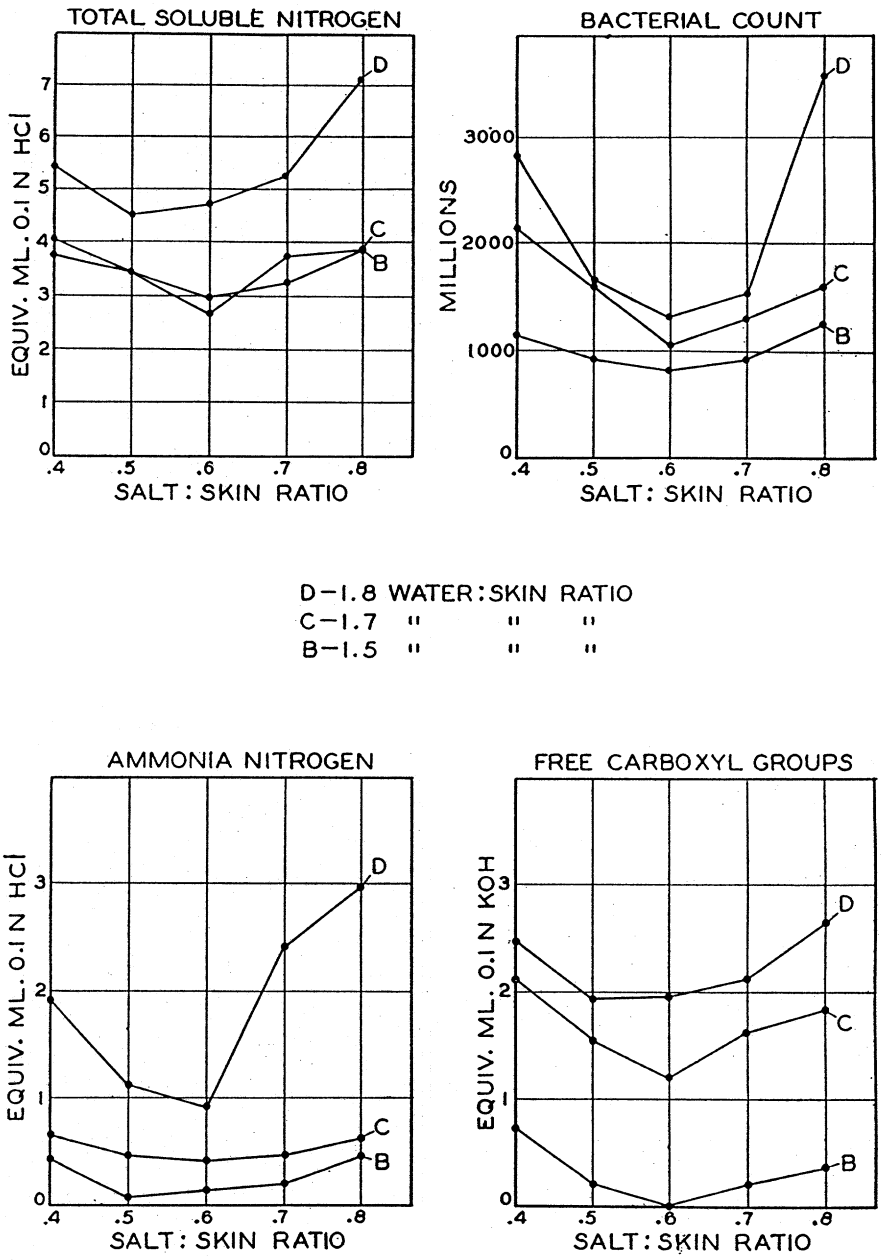

FIGURE II. Effect of increasing salt to skin ratio on bacterial count, free carboxyl groups, total soluble nitrogen, and ammonia nitrogen with resalted calfskin.

TABLE III

Increase in total soluble nitrogen, ammonia nitrogen, carboxyl groups, and bacterial count in the incubated samples over the unincubated controls.

(Expressed on the basis of 1 gm. of salt-free, moisture-free skin prior to resalting.)

Approximate Ratio of Salt to Skin	Cured Skin—A Approximate Ratio of Water to Skin = 1.3 Total Soluble Nitrogen	Ammonia Nitrogen	Carboxyl Groups	Bacterial Count	Cured Skin—B Approximate Ratio of Water to Skin = 1.5 Total Soluble Nitrogen	Ammonia Nitrogen	Carboxyl Groups	Bacterial Count
	ml. 0.1N HCl	ml. 0.1N HCl	ml. 0.1N KOH	millions	ml. 0.1N HCl	ml. 0.1N HCl	ml. 0.1N KOH	millions
4	.00	0.14	.01	0	3.67	0.44	0.72	1,194
5	+.02	0.09	.00	0	3.39	0.06	0.24	993
6	−.02	0.09	.01	0	2.66	0.15	0.00	1,029*
								891
7	−.02	0.07	.01	0	3.76	0.22	0.24	944
8	.00	0.10	.01	0	3.85	0.47	0.36	1,259

Approximate Ratio of Salt to Skin	Cured Skin—C Approximate Ratio of Water to Skin = 1.7 Total Soluble Nitrogen	Ammonia Nitrogen	Carboxyl Groups	Bacterial Count	Cured Skin—D Approximate Ratio of Water to Skin = 1.8 Total Soluble Nitrogen	Ammonia Nitrogen	Carboxyl Groups	Bacterial Count
	ml. 01.N HCl	ml. 0.1N HCl	ml. 0.1N KOH	millions	ml. 0.1N HCl	ml. 0.1N HCl	ml. 0.1N KOH	millions
.4	4.04	0.66	2.11	2,151	5.42	1.90	2.47	2,762
.5	3.48	0.46	1.53	1,806*	4.56	1.11	1.91	1,515*
				1,619				1,695
.6	2.91	0.41	1.24	1,046	4.75	0.91	1.91	1,310
.7	3.23	0.46	1.61	1,505*	5.28	2.41	2.15	1,326*
				1,307				1,533
.8	3.97	0.63	1.82	1,624	7.15	2.97	2.68	3,636

*Top figure is the average gain in bacterial count; bottom figure is the average gain in bacterial count as corrected by the addition or subtraction of the probable error for use in plotting figures 1 and 2.

the normal amount adsorbed by the skin in an adequate green-salting cure will increase the efficiency of preservation. Resalting with larger amounts of salt may prove harmful.

In Figure I the increase in total soluble nitrogen, ammonia nitrogen, bacterial count, and free carboxyl groups with samples containing different amounts of excess salt is plotted against the initial moisture content of the four cured skins. In this figure the curves are strikingly similar regardless of the amount of added excess salt. This illustrates further the ineffectiveness of excess salt as a dehydrating agent for salt-cured skin. In this figure the curves are identified by the numbers 1 to 5, inclusive, to correspond to the identification of the samples in Table II. These numbers refer to the different salt to skin ratios as shown in Table III. If the values in Figure I were plotted against the final percentage of moisture after the addition of salt as given in Table II, no correlation would be found between increases in soluble nitrogen, ammonia nitrogen, free carboxyl groups, and bacterial counts and gain in moisture content.

The results given in Table III for the samples from skins B, C, and D are plotted in Figure II to show the effect of adding different amounts of salt in

excess of that adsorbed by the skin during curing, on increases in total soluble nitrogen, ammonia nitrogen, bacterial count, and free carboxyl groups. Although, as might be expected from the influence of moisture shown in Figure I, there is considerable difference between the levels of the curves for the samples having different water-to-skin ratios, the configuration of the curves is the same in general for all three samples. A decrease in total soluble nitrogen, ammonia nitrogen, number of bacteria, and free carboxyl groups is shown as the salt-to-skin ratio increases to approximately 0.6. With ratios of salt to skin of 0.7 and 0.8, there is an increase in total soluble nitrogen, ammonia nitrogen, the number of bacteria, and free carboxyl groups.

Discussion and Conclusions

Similar studies with additional skins in which only individual chemical changes were considered or in which only bacterial counts were made indicate that the data reported herein can be considered as representative.

Koppenhoefer and Somer[3] recently published studies of the effect of particle size of salt on the extent of changes in salt-cured steer hides, in which they present curves showing changes in the amounts of volatile nitrogen and free fatty acids with increases in the particle size of salt used in curing. The similarity of the configuration of their curves to those shown herein on the influence of increasing the salt-to-skin ratio above 0.4 on bacterial counts, total and ammonia nitrogen and also free carboxyl groups is striking, particularly if one bears in mind the fact that rate of solubility decreases as salt particle size increases. It is possible, therefore, that their results might also be interpreted as an effect of the relative amounts of excess salt remaining on the skin after a salt-to-skin ratio of 0.4 had been attained. This possibility seems fully as logical as the explanation offered, which is based on a case-hardening effect of fine salt and a slow rate of solubility for coarse salt.

The decrease in preservative efficiency with increase in the ratio of salt to skin to 0.7 and 0.8 or in the amount of excess solid salt above approximately 5 or 6 per cent on the weight of the cured skin might be explained in the light of findings by Clayton[4], Lochhead[5], Robertson[6], Stuart and James[7], and Stuart[8]. All these investigators have found that halophilic bacteria are stimulated in their growth by increases in the sodium chloride concentration above a minimum of approximately 3 molar. An excess of unadsorbed and uncombined salt capable of taking up 1 or 2 equivalent molecules of water would increase the salt concentration of the soluble phase through the removal of water. Thus, as long as there is enough free moisture present to support microbial growth, a large excess of salt might be expected to stimulate the growth of halophilic bacteria. The stimulation of growth would be accompanied logically by increased physiological activity, resulting in a more rapid digestion of skin proteins.

Hausam[9] has stated that skins salted with either too little or too much

salt are subject to damage during curing. Although the present work cannot be considered in a strict sense as a curing study, the results might be cited as experimental proof of Hausam's contention that damage can be brought about through the use of too much salt.

The changes shown in these studies are undoubtedly due to halophilic bacteria. Thus, the interpretation of the results given to conditions existing in commerce in the curing and storage of skin must take into account the physiological peculiarities of this group of organisms. These bacteria have lag phases of from ten days to two weeks or even longer, before they normally start to grow. This interval of time undoubtedly would be long enough to permit the thorough permeation of the skin with salt, even when crude solar salt or old, used salt, both of which are heavily contaminated with halophilic bacteria, are used, before changes of the type reported herein should start to occur. With new uncontaminated salt, or salt free from halophilic bacterial types, there would be a much longer period of time before such changes should start to occur for the development of halophilic bacteria from the normal contamination found on skin, in dung and soil usually requires periods of 30 to 40 days at least and frequently 60 to 90 days, depending upon the availability of nutrients in the substrate. Thus, the results given are much more applicable to the storing of old cured skins upon which a halophilic flora has become established than to the curing of freshly flayed skins.

There is an interesting connection between the results found in these studies and reports from one large exporter of cured skins. This particular concern resalts calfskins for export with 6 per cent of fine salt on the basis of the cured weight of the skin as received. Whether this amount was selected because experience has shown that it is the most efficient quantity to use or whether it was selected from the standpoint of the economic use of salt is not known. Small scale laboratory resalting experiments have shown that this is the smallest amount of salt that can be used on cured calfskins and obtain uniform distribution over the entire flesh side.

The results suggest that after a curing period long enough to allow for the complete penetration and dehydrating action of the salt, hides and skins probably can be preserved more effectively by storing them in bundles rather than in beds containing large excesses of salt.